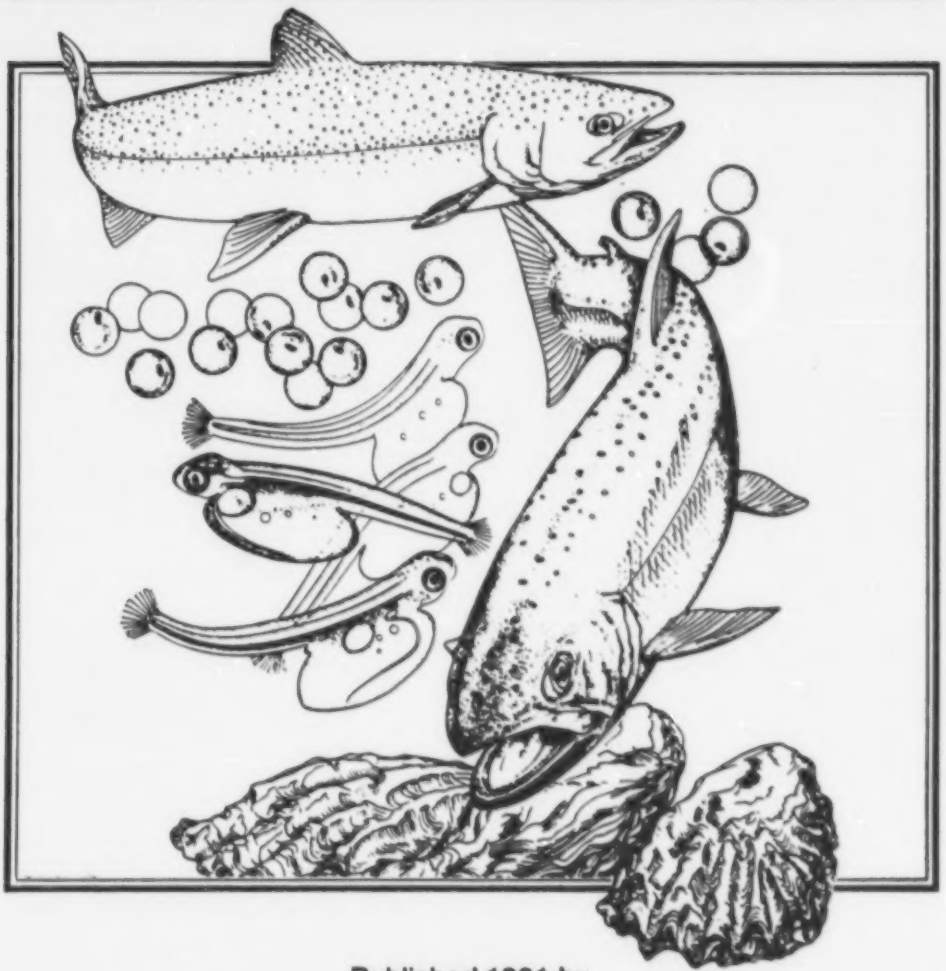
Aquaculture Industry Development Report

# Third British Columbia Fish Nutrition Research Seminar Current Issues in Salmonid Nutrition and Feeding

## REVIEW PAPERS AND SUMMARY



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November 15-17, 1989
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**Note:**

This report includes four of six review papers presented at the meeting plus a very brief summation of the main points brought up in discussion of the topic areas. The review papers are presented here as they were submitted with no editing. The program and a list of speakers and participants have been included so that they can be contacted to discuss current research if needed.

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# ASCORBIC ACID - KNOWN AND HYPOTHESIZED FUNCTIONS, EFFECTIVE DIETARY SUPPLEMENTATION

**Tom Lovell**

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# Ascorbic Acid - Known and Hypothesized Functions, Effective Dietary Supplementation

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## Abstract

Feeding experiments were done with channel catfish to demonstrate the relative vitamin C activity of two heat-stable compounds, L-ascorbyl-2-phosphate (AAP) and L-ascorbyl-2-sulfate (AAS). Results showed that AAP has equimolar vitamin C activity as L-ascorbic acid (AA) for growth of channel catfish, and that AAS has same activity but only about 7% of that of AA or AAP. Higher tissue levels of ascorbic acid in liver and kidney in fish fed (AAP) indicate that more ascorbic acid was absorbed when AAP was fed than when AA was fed.

Research on dietary level of ascorbic acid, below and above the normal growth requirement, and resistance to bacterial infection in fish is reviewed. Dietary deficiency of ascorbic acid increases mortality and reduces immune responses in channel catfish and rainbow trout. Feeding elevated doses, 10 to 100 times the minimum growth requirement, of ascorbic acid to small fish in the laboratory, dramatically reduced mortality rate in channel catfish and rainbow trout challenged with *Edwardsiella ictaluri* and *Vibrio anguilarum*, respectively. Feeding megadoses of ascorbic acid to channel catfish under practical culture conditions produced less dramatic immune responses; however, the LD for concentration of *E. ictaluri* cells injected into channel catfish increased as dietary dose of ascorbic acid increased. Serum, liver, kidney and skin concentration of ascorbic acid

ceased to increase when dietary dose exceeded 500 to 1000 mg/kg, indicating saturation of metabolic pools in channel catfish and that megadose feeding need not exceed this dose range.

This report covers two subjects: I, Vitamin C activity for L-ascorbic acid, L-ascorbyl-2-sulfate, and L-ascorbyl-2-phosphate Mg for channel catfish; and II, Vitamin C and resistance to bacterial infection

## I.

Vitamin C is an important nutrient in fish feeds. While most land animals don't require it in their diet, most fishes are extremely sensitive to a deficiency which not only reduces growth rate but causes physical deformities (crooked backs, etc.), slows wound healing, and reduces resistance to infections, environmental contaminants (nitrites, chlorinated hydrocarbons, etc.) and other stresses.

L-ascorbic acid is the vitamin C sources used in commercial catfish feeds. It is sensitive to oxidation, and heat and moisture in feed processing destroy a significant amount. Coating ascorbic acid with various compounds offers some but limited protection during processing. Results of a study we made several years ago, where stability of ascorbic acid was evaluated at three extrusion mills and two steam pelleting mills, showed that the half-life (time required for 50% to be lost) of vitamin C in dry catfish feeds during storage is less than 90 days.

Because of the large losses of ascorbic acid during processing and storage, new compounds with vitamin C activity have been investigated for use in aquatic feeds. For a compound to replace ascorbic acid, information must be obtained on its efficacy (does it have the same function as ascorbic acid) and stability during processing and storage. In addition, the compound must have government approval for use in feeds for food fish. Of course, a replacement for ascorbic acid in aquatic feeds must also have economic benefit.

Compounds that have been presented for use in fish feeds are phosphate and sulfate derivatives of ascorbic acid. Ascorbic acid-sulfate is relatively stable during processing and storage and has been reported to have significant vitamin C activity for salmonids. It also has vitamin C activity for channel catfish, but how much is not known. Phosphorus derivatives of ascorbic acid have been investigated for use in aquaculture feeds and shown to have vitamin C activity for several species and good stability in processing and storage of dry and moist feeds. The relative vitamin C activity of these ascorbic acid derivatives for various species has not been determined.

Two products with potential vitamin C activity were evaluated against L-ascorbic acid with channel catfish at Auburn University. L-ascorbyl-2-phosphate Mg (AAP), L-ascorbyl-2-sulfate (AAS) and L-ascorbic acid (AA) were fed at various dietary doses to channel catfish under laboratory conditions. On April 26, 1988, 39 aquaria (50-liter) with flowing water (29±2°C) were each stocked with 21 full sibling channel catfish (8g average size). The fish were fed a basal purified diet (Table 1) with no vitamin C for two weeks. On May 11, three aquaria were randomly assigned to each of 13 experimental diets. They were fed a control diet (no vitamin C) and diets containing the three compounds with vitamin C activity at four concentrations. The compounds were added to the diets on an equimolar ascorbic acid basis at concentrations of 11, 22, 44 and 132 mg/kg. The fish were fed the assigned diets twice daily to satiation.

One July 18, after eight weeks on the experimental diets, the fish were weighed and examined for abnormal fish. Fish were randomly collected from each treatment for histological examination. After 14 weeks, numerous anomalies and mortalities had occurred in some treatments and the feeding trial was terminated. The fish were weighed, examined for gross anomalies, and blood samples and tissues were taken for hematocrit measurement, histological examination, collagen measurement, and ascorbic acid determination.

The analytical procedure for ascorbic acid determination in the feed and in the fish tissue was by HPLC with an electrochemical detector, using a modification of the procedure of Wang et al. (J. Assoc. Off. Anal. Chem. 71(6):1158 (1988)). This involved extraction with meta phosphoric acid and dithiothreitol (DTT) and diluting with perchloric acid for chromatographing. For AAP, the extract was adjusted to pH 4.8 with Na acetate and digested with yeast and acid phosphate at 37° C, then diluting with perchloric acid and chromatographing. The procedure for measuring AAS involved conversion of AAS to AA by solvolysis in dry acidic methanol at 25° C, and subsequently measuring AA as described above (Wang, 1982. Master's thesis, Kansas State University).

Results of weight gain, gross deficiency signs, blood hematocrits, and liver and kidney levels of ascorbic acid are reported here. Other results will be reported when analyses are completed. Losses of vitamin C activity during feed processing are preliminary and further evaluations are being made.

Diets supplemented with the lowest concentration of l-ascorbic acid (AA) and ascorbic acid phosphate (AAP) provided normal growth by the fish (Figure 1), indicating equal molar ascorbic acid activity for growth. None of the concentrations of ascorbic acid sulfate (AAS) supported normal growth. Regression of fish growth on dietary concentration of AAS allowed determination of the concentration of AAS that produced the same growth as l-AA. This indicated that the molar equivalent activity of AAS was 7% of that of AA for growth.

There were no gross signs of vitamin C deficiency in any of the fish fed AA or AAP (Figure 2). Profound spinal deformities were found in fish fed the control diet and in fish fed 11, 22 and 44 mg/kg of AAS. percentage of fish with spinal deformities increased. There were no deformities among the fish fed 132 mg/kg of AAS. These data agree with the growth data.

Hematocrits (red blood cell volume) were equal for fish fed AA and AAP, but lower for fish fed the control and the AAS diets containing 44 mg/kg and less (Figure 5). Hematocrit was normal for fish fed the highest level of AAS.

Liver and kidney concentrations of ascorbic acid increased as dietary ascorbic acid increased with fish fed AA and AAP, but not with fish fed AAS where only traces of tissue ascorbic acid could be found (Figures.3, 4). Higher tissue concentrations were found in fish fed AAP than in those fed AA, especially at higher dietary levels. This may indicate that ascorbic acid in the diet was more stable in the AAP form, or that the phosphate moiety enhanced the absorption of ascorbic acid from the intestine. Perhaps the phosphate form was absorbed more slowly but more efficiently than free AA. Possibly some of the AAP was absorbed intact and the phosphate protected the asorbic acid from degradation in the tissue during storage (-85°C) and extraction.

We have not gotten satisfactory measurements of AA concentration in the purified diets. We have gotten better recoveries with practical feeds. Percentages of ascorbic acid recovered in extrusion processed feeds containing AA and AAP were 59 and 70, respectively. This is lower than anticipated for AAP. We feel that our analytical procedure for ascorbic acid, which gives satisfactory results for tissue analysis, needs further refinement for feed materials. We will make further measurements of extrusion losses of AAP.

These results indicate that AAP has equimolar vitamin C activity as AA for growth of channel catfish and that AAS has vitamin C activity, but at a much lower rate than the other ascorbic acid compounds. AAP may be superior to l-AA for maintaining tissue levels of AA.

## II.

Dietary deficiency of most of the vitamins has increased sensitivity of animals to bacterial infection (1). Thus, it should be expected that animals which do synthesize ascorbic acid will have a reduction in immunocompetence when deprived of this vitamin in the diet. A number of reports have found this to be true for guinea pig and primates, which have a dietary requirement for this vitamin. This is discussed in several reviews (1, 2,3) and will not be covered in this report which will be limited to fish, most of which have limited ability to synthesize ascorbic acid.

In early catfish feeding studies, Lim and Lovell (4) reported higher mortalities from bacterial infections in fish in ponds that were fed diets devoid of ascorbic acid than in fish fed ascorbic acid. Blazer (5) found in controlled environment studies that rainbow trout fed diets marginally deficient in ascorbic acid showed depression of the nonspecific resistance measurements, iron binding capacity and phagocytosis, when the fish were challenged with Yersinia ruckeri. Later studies showed that channel catfish challenged with *Edwardsiella ictaluri* (6, 7) and rainbow trout challenged with *Vibrio anguilarum* (8), by injection and immersion, had markedly higher mortality rates when they had previously been deprived of dietary ascorbic acid.

We were interested in the effects of feeding elevated levels of ascorbic acid, levels above that required for normal growth and prevention of overt deficiency signs, on resistance to bacterial infection in channel catfish. This was because beneficial effects of elevated levels of ascorbic acid on resistance to infection have been reported for human (9), and guinea pig (10). Also, the dietary requirement for ascorbic acid by channel catfish varies with metabolic function or criteria evaluated. For example, minimum requirements for growth, prevention of scoliosis or lordosis, and maximum rate of wound repair are different (6, 7, 11); thus, higher than normal dietary level may be beneficial subsequent to bacterial infection. In our initial study (6) channel catfish fingerlings were fed semipurified

diets containing 0-150 mg/kg of ascorbic acid for 14 wk and subsequently, at two temperature regimes, infected with the pathogenic bacterium *Edwardsiella ictaluri*. Mortality rates were determined 96 h after injection. A supplemental ascorbic acid level of 30 mg.kg of diet was sufficient for normal growth and for prevention of deficiency signs such as lordosis, scoliosis, and a reduction in bone collagen formation. However, increased resistance against infection was provided when the level of supplemental ascorbic acid was increased to the highest dietary level, 150 mg/kg, at a water temperature of 23°C. At 33°C, increasing the supplemental level of ascorbic acid had significantly less effect on resistance against infection. The difference in response at the two temperatures indicates that the ascorbic acid requirement for resistance to infection is possibly higher when channel catfish are infected at a temperature near optimum for the natural resistance mechanisms of the fish.

These results suggested that channel catfish require higher than normal dietary levels of ascorbic acid for certain physiological functions, such as response to bacterial infection, or that megadose levels have some pharmacologic effect in increasing resistance to infection. A subsequent study (7) was designed to evaluate effects of zero to megadose levels of dietary ascorbic acid on resistance to *E. ictaluri* infection, the specific immune response, antibody production, and on the nonspecific defense systems, serum complement activity and phagocytosis, in channel catfish. Channel catfish fingerlings were fed purified diets containing 0 to 3000 mg/kg of ascorbic acid until external signs of scurvy were seen in the fish fed the ascorbic acid-deficient diet. At this time, resistance to bacterial infection, antibody production, complement activity and phagocytic activity were assessed for fish from the various dietary treatments. Mortality rates of fish experimentally infected with *Edwardsiella ictaluri*, the bacterium causing enteric septicemia in channel catfish, decreased with increases in dietary ascorbic acid doses, ranging from 100% for fish fed the ascorbic acid-deficient diet to 15% for fish fed 300 mg ascorbic acid per kilogram of diet.

Antibody response to *E. ictaluri* antigen, hemolysis of sensitized sheep erythrocytes by complement activity and phagocytic engulfment of *E. ictaluri* by peripheral phagocytes were each impaired in fish fed the diet without supplemental ascorbic acid; intracellular bactericidal activity of the phagocytes was not affected by ascorbic acid deficiency. There were no differences in antibody production, complement activity, or phagocytic activities among fish fed diets containing 30-300 mg ascorbic acid/kg of diet. However, the dose level of 3000 mg of ascorbic acid/kg of diet significantly enhanced antibody production and complement activity.

Similar responses were also found with rainbow trout (8). Fingerlings (initial weight, 1 g) were fed purified diets containing 0,100,500,1000, or 2000 mg of ascorbic acid per kg of dry diet. After 18 weeks, representative fish from each lot were challenged with pathogenic *Vibrio anguilarum* (strain NCMB6) to measure susceptibility and/or resistance to the pathogen. Two types of challenge were used, peritoneal injection or bath immersion in measured concentration of pathogen. Survival was directly related to supplemental level of ascorbic acid in the dietary treatment. A second test with immunized fish indicated that the primary early defense was not by humoral antibody synthesis. A secondary defense system by cell mediated immunity or by non-specific factors, which was dependent upon ascorbic acid intake, was responsible for rapid defense against this pathogen. After ten weeks post immunization, however, high levels of ascorbic acid dietary intake promoted late humoral antibody activity, and enhanced survival of fish fed higher than recommended requirements for vitamin C ( 100 mg/kg dry diet).

The optimum dietary dose level of ascorbic acid for resistance to infection in channel catfish was apparently between 300 and 3000 mg/kg. On the assumption that this optimum level would be the minimum dietary dose of ascorbic acid that would cause body pool saturation, a study was conducted to establish the regression of tissue levels of ascorbic acid on dietary dose (12). After 46 days of feeding, tissue levels of ascorbic acid

generally increased until 500 mg of ascorbic acid/kg feed was fed, then plateaued. Ascorbate-2-sulfate, an assumed storage form of ascorbic acid, was present in the skin and liver in significant amounts but was not affected by dietary level of ascorbic acid. These results suggest that tissue ascorbic acid will increase with dietary dose until the metabolic pools are saturated, which occurs at about 500 mg/kg of diet, and the excess dietary ascorbic acid is not stored as ascorbate-2-sulfate but probably excreted. Supplementing catfish practical diets with 500 to 1000 mg ascorbic acid/kg, instead of 3000 mg/kg as previously assumed, would allow saturation of the ascorbic acid metabolic pools and any protection against bacterial infection which megadoses of ascorbic acid may provide.

Effects of feeding megadoses of ascorbic acid to channel catfish under practical type conditions were evaluated. In one study (12) channel catfish (*Ictalurus punctatus*), averaging 21.8 g, were fed experimental diets with six different levels of ascorbic acid (0, 100, 500, 1000, 2000, and 4000 mg/kg diet) in earthen ponds for 46 days. On May 9, when water temperature reached 25°C, 250 fish were seined from each pond. They had grown to 58.3 g with no difference among treatment weights or signs of scurvy. One hundred of the fish from each treatment were infected with *Edwardsiella ictaluri* by immersion in a suspension of $10^3$ cells/ml. The fish were reinfected 9 days later because mortalities were slow to occur. Mortalities from the infection were low and revealed no effect of ascorbic acid deficiency or various supplemental levels. Reasons for this result may be that the effect of dietary ascorbic acid was masked by a low infection rate, by probable exposure of fish to the pathogen prior to the experiment, by the ascorbic acid from natural pond food, or by the short feeding period.

In this study there was no effect of dietary dose level or deficiency of ascorbic acid on antibody titers. However, after IP injection challenge with virulent *E. ictaluri* at high bacterial concentration ( $10^7$ and $10^9$ cells/ml), the mortality rates of fish increased with

decreasing supplemental ascorbic acid in the diets (13). The $LD_{50}$ doses increased with increasing ascorbic acid levels.

Robinson[1] (unpublished) fed commercial catfish feeds containing a normal supplement (100-150 mg/kg) and an elevated supplement (1300 mg/kg) of ascorbic acid to channel catfish in cages suspended in a pond for 30 days, then transferred the fish to a pond with a heavy epizootic of *E. ictaluri*. After 2 weeks, there was a significantly lower mortality among the fish fed the high dose of ascorbic acid; 44% versus 66% for the control.

The pond experiments indicate that fish grown under more practical conditions do not respond as dramatically to high dose feeding of ascorbic acid as do small fish under laboratory conditions, but they do substantiate the enhanced resistance to bacterial infection by channel catfish fed higher than normal doses of the vitamin.

Table 1. Basal purified diet[1]

| Ingredient | Amount % |
|---|---|
| Vitamin-free casein | 32.6 |
| Gelatin | 9.4 |
| Dextrin | 33.05 |
| Cellulose | 10.0 |
| Carboxymethylcellulose | 3.0 |
| Soybean oil | 4.0 |
| Cod-liver oil | 3.0 |
| Mineral mix | 4.0 |
| Vitamin C-free vitamin mix | 1.4 |

[1] Vitamin C sources were added at various diet concentrations to replace cellulose.

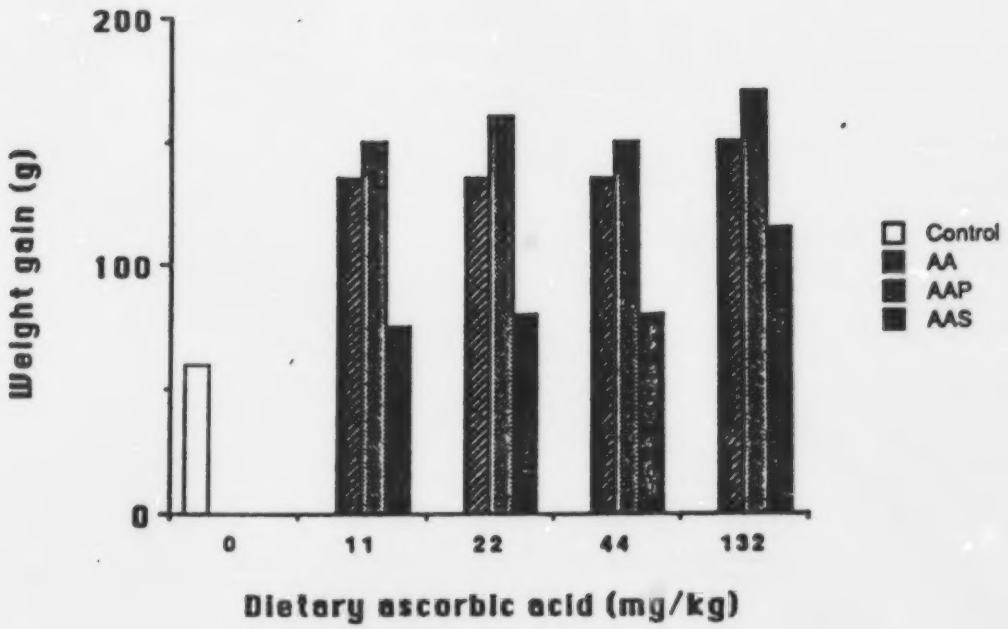
200
100
0
Weight gain (g)
0
11
22
44
132
Dietary ascorbic acid (mg/kg)
Control
AA
AAP
AAS

Figure 1

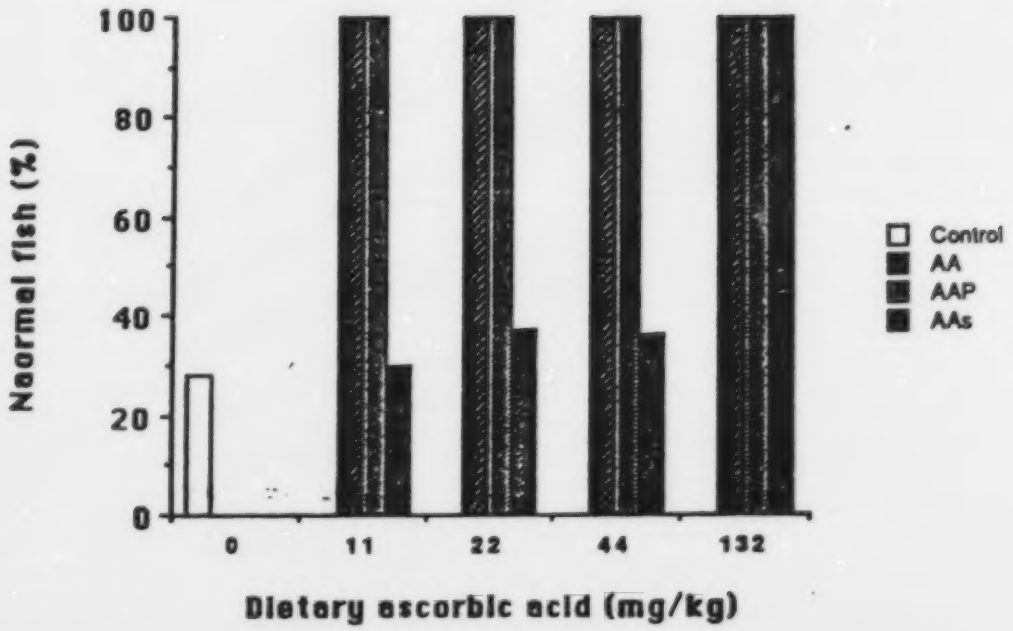
100
80
60
40
20
0
Naormal fish (%)
0
11
22
44
132
Dietary ascorbic acid (mg/kg)
Control
AA
AAP
AAs

Figure 2

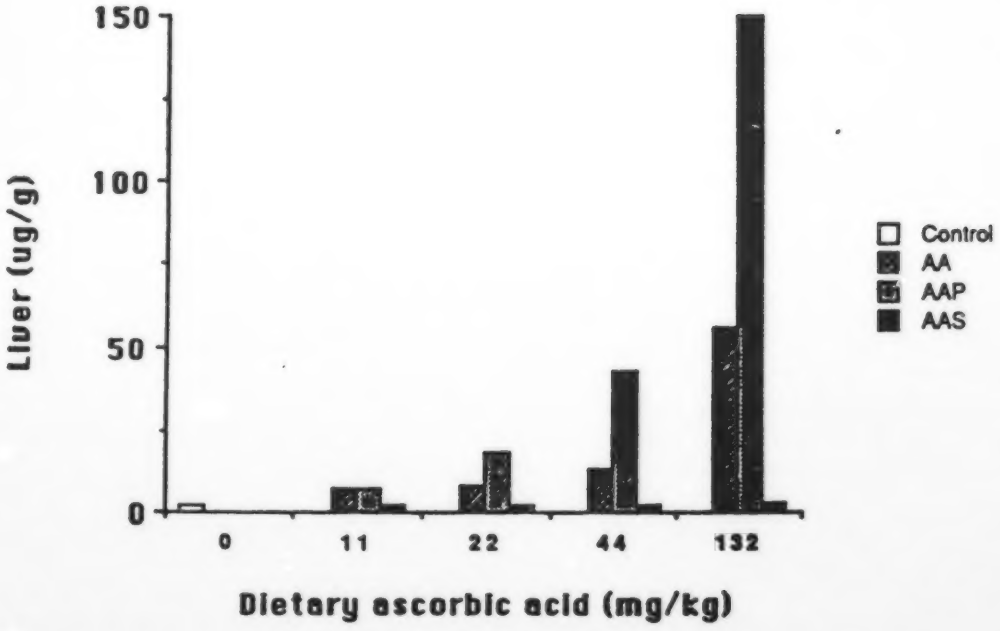
150
100
50
0
Liver (ug/g)
0
11
22
44
132
Dietary ascorbic acid (mg/kg)
Control
AA
AAP
AAS

Figure 3

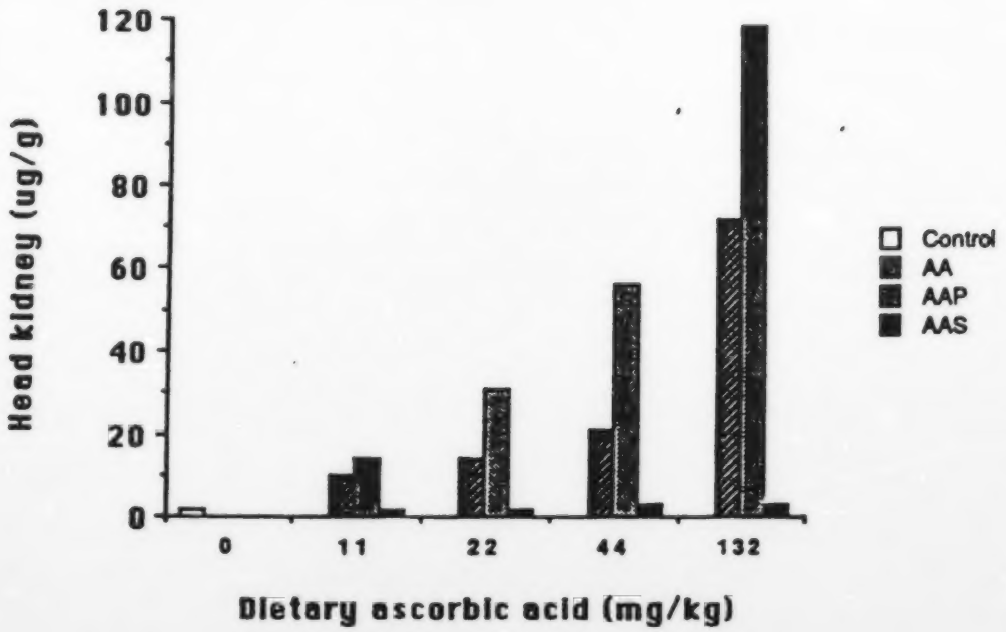
120
100
80
60
40
20
0
Head kidney (ug/g)
0
11
22
44
132
Dietary ascorbic acid (mg/kg)
Control
AA
AAP
AAS

Figure 4

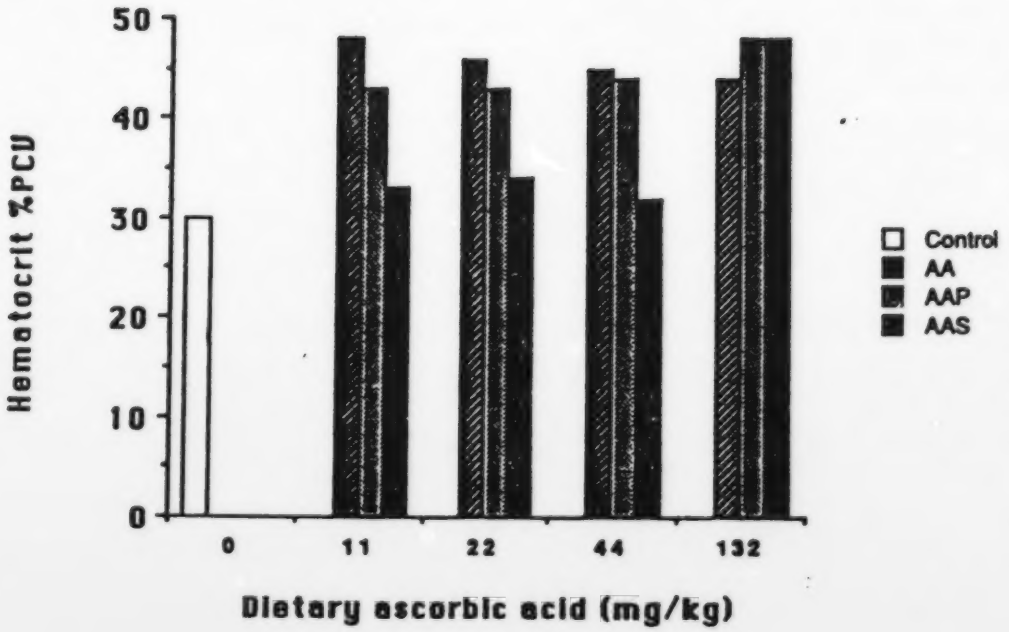
Hematocrit %PCV
50
40
30
20
10
0
0
11
22
44
132
Dietary ascorbic acid (mg/kg)
Control
AA
AAP
AAS

Figure 5

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## Antibiotic Use in Fish Culture

A discussion of use of antibiotics in fish must begin with a discussion of their use in terrestrial animals. The in-feed antibiotic era began when the dried fermentation mash of Streptomyces aureofaciens, the organism responsible for chlortetracycline, was used as a source of Vitamin B12. It had previously been found that the so-called "animal protein factor" found in liver could be replaced by Vitamin B12, thus the search for a source of that nutrient. In using the fermentation mash, Stokstad et al (1) found that the growth of chicks was much greater than could be accounted for by Vitamin B12. Investigation soon proved that the cause of this extra growth was, in fact, the chlortetracycline remaining in the mash (2).

Around 1950, Stokstad and Jukes (3) were able to demonstrate substantial improvements in gains and feed conversion ratios using diets supplemented with chlortetracycline. While antibiotics had previously been used therapeutically by injection or intramammary infusion, this was the beginning of their use in feed (4). Soon afterward, many other antibiotics, including oxytetracycline, bacitracin, and penicillin were found to enhance growth and feed efficiency. As years went by, specialized agricultural products were developed, including such compounds as tylosin, carbadox, the ionophores (monensin and salinomycin), and bambermycins. Table 1 presents many of the antibiotics and antibacterial compounds now used in animal agriculture.

The development of in-feed antibiotic use in land animals has evolved to today's standards in a sound and logical manner. Preventive and growth-promotant programs are scientifically based and well controlled. Today's regulatory procedures at the Bureau of Veterinary Drugs, Health Protection Branch ensure the human and use-animal safety, and the efficacy of the products, as well as proper manufacturing and quality control procedures. Agriculture Canada maintains strict inspection over food products, including monitoring for tissue residues. A safe, wholesome product is therefore assured to the public.

The situation in fish culture, and especially farmed salmon, is somewhat different. Because of the rapid growth of this industry, pharmaceutical manufacturers, feed manufacturers, regulatory officials, and producers have been scrambling to answer all the questions being asked.

The very nature of the fish presents us with many special problems which need to be addressed. While we can look at the history of antibiotic use in land animals as a guide, we must be aware of the differences in marine and aquatic animals and their environment as we continue to develop antibiotic programs for use in farmed fish.

## Antibiotic Use in Fish - Treatment of Disease

Aquaculture is not new to North America and certainly not new to the world. We are all well aware of the ancient Chinese culture of carp for food. In the U.S., trout farming began as early as 1853 (3). In Canada, or at least in British Columbia, aquaculture began with an oyster farm in 1912. Trout culture started in 1950 and salmon in the 1970's (6). Intensive production of fish and, particularly, intensive production of salmon reared in sea pens, is a relatively modern undertaking. Under these conditions, disease control may well be the limiting factor to optimum production. The use of antibiotics as a tool in achieving that disease control dates back some 40 years, to a period just after the launch of the "antibiotic era" in land animal production.

Use of modern antibacterials against systemic diseases of fish was reported as early as 1939, when sulfanilamide was tested, but found to be toxic. In 1948, sulfamerazine was evaluated and found to be both safe and efficacious against furunculosis. About the same time, nitrofurans were first evaluated, with furazolidone later being developed as a suitable medicament. Furanace, a later nitrofuran derivative, has been developed specifically for use in fish, owing to its low toxicity, reasonable eficacy, and propensity to be readily absorbed topically (7).

One of the earliest reports of the evaluation of antibiotics for the treatment of a disease came in 1951 when Snieszko et al (8) demonstrated the successful treatment of ulcer disease in trout using oxytetracycline and chloramphenicol. These same authors reported a year later on the antibiotic treatment of ulcer disease and furunculosis in trout (9). By 1957, Snieszko had presented a review of antibiotic use in fish to the American Chemical Society. This review, which was published two years later (10), summarized his work with oxytetracycline, chlortetracycline, and chloramphenicol, those antibiotics being judged most practical based on stability, cost, and availability. He reported that antibiotics provided in the feed, already established by that time as the most-used means of drug delivery, was efficaceous against various diseases including ulcer disease, furunculosis, and kidney disease.

In the United States, it was not until 1967 that the first antibacterial, sulfamerazine, was registered for use in fish. This arose following a concerted effort by the U.S. Fish and Wildlife Service beginning in 1963. A similar effort, in cooperation with Pfizer Inc., resulted in the registration of Terramycin in 1970 (11). More recently, the poteniated sulfa, sulfadimethoxine - ormetroprim, was the

third antimicrobial to be licenced (12). Here in Canada, it was only recently, that, due to the rapid rise in the need for antibacterials for fish culture, paralleling the exploding salmon farming industry, Terramycin was cleared through the Health Protection Branch for use in fish.

In Europe, a number of antibiotics have found a place in fish culture. In the U.K., oxolinic acid, oxytetracycline, and a potentiated sulfonamide (sulfonamide plus trimethoprim) are licenced for use in fish (13). A Norwegian publication by Grave (14) reports the intensity of the use of antibiotics during the period 1980 to 1986. The commonly used antibacterial agents included oxytetracycline, nitrofurazolidone, trimethoprim-sulfadiazine, and sulfamerazine. The use of these compounds peaked in 1984 when 87% of all farmed fish received treatment. By 1986, this figure had fallen back to 40%. Table 2 gives the antibacterial agents used in fish feed throughout the world.

## Antibiotics Use in Fish - Growth Promotion

The dawn of in-feed antibiotic use in land animals was heralded by the discovery of their growth-promoting capabilities. It follows, therefore, that a quest for similar properties in fish would be undertaken. By the time of Snieszko's 1959 review, several studies had been conducted evaluating many antibacterials, including chlortetracycline, oxytetracycline, chloramphenicol, penicillin, and bacitracin for improvement of growth in fish. There was found to be no such activity (10).

The growth-promoting effect of antibacterials in homeotherms is well-documented. Although the precise mechanism of action of all antibacterials in enhancing growth is not completely understood, it is almost certain that it is due to their antibacterial activity. This may be due to nutrient-sparing, as has been demonstrated with carbadox, alteration of the usable metabolites due to bacterial action on foodstuffs, as exemplified by the ionophores, or the control of subclinical disease, as seen with many antibiotics (15). It is also very likely that this is the reason antibacterials do not enhance growth in fish.

The bacterial flora of fish is not comparable to that of land animals, or at least of homeotherms. Such populations in fish are not constant and may even by absent (10). A report by Austin and Al-Zahrani (16) indicated that there was a progressive decline of bacteria along the digestive tract. These bacteria were present in some abundance in the intestinal contents but did not appear to colonize the gut. The gut flora resembled that of the environment, but in

somewhat greater concentration. Antibacterial treatment of fish caused a change in the numbers of bacteria in the gut and the make-up of the flora. Feeding of antibiotics to warm-blooded animals, by comparison, results in a change in the proportions of the microflora, but causes minimal changes in the total numbers. These changes are often beneficial to the host animal (15). It would therefore appear that the gastro-intestinal microflora of fish provides little in the way of nutrition to the fish and the change in that population subsequent to antibacterial treatment has no beneficial effect on the growth of the fish.

## Feed as a Drug Delivery Vehicle

When a drug is required by a patient, it must be delivered to that patient in some way. If the patient is human, delivery is quite easy. Drugs can be given by injection or by mouth in precise amounts. If the patient is an animal, it is only slightly more difficult as the same routes may be used with equal precision but not always with the same amount of cooperation. If the patient is a large group of animals, the difficulty of delivering that drug is multiplied several-fold. Injection of each is tedious, but possible. Water delivery may be used. Medication given via the feed is a third alternative which is superior only in its practicability. It is the least desireable method in that it provides the least precise method of dosing. And that brings us to our patient under discussion - the large group of fish. While injection of each specimen is possible it would be extremely tedious and traumatic - for both the patient and the caretaker. That leaves us with only the least desireable option - the delivery of medication in the feed.

The use of feed as a drug-delivery vehicle has some benefits but many shortcomings as well. Feed is a practical means of getting medication into fish, particularly if one's goal is prophylactic, as all fish will be generally healthy and eating. Fortunately, fish are voracious eaters and we can be fairly well assured that the medication is getting into them.

If, on the other hand, our group patient is sick, we can be assured that, at least in some individuals with advanced disease, the drug is not adequately getting into them. Some individuals may be too weak to compete for feed, others may not be eating. Certainly, at-risk individuals within the group will benefit from therapy, but management methods must be considered that will address the sick individuals.

The use of feed as drug-delivery system for fish presents us with a number of additional concerns which need to be addressed. The aquatic environment of fish, as compared to the traditional agricultural environment, is unique and adds even further problems.

During the development of any feed additive, antibiotics included, the stability of the compound during feed manufacture is evaluated. This involves assaying feed samples taken before and after the pelletting process. Any product on the market, therefore, can reasonably be expected to be stable during the pelletting process, under normal pelletting conditions.

Many fish feeds are extruded or moist, however. Questions have been raised about the stability of antibiotics under the conditions of manufacture of these feed types. Conditions under which feed is extruded are variable, but at least some work has been done to examine the stability of oxytetracycline in extruded feed. The preliminary results of this work will be presented by Jack Markert in a later presentation.

The leaching of drugs from the feed is yet another concern unique to aquatic species. Fribourgh and others (17) presented a study in 1969 which addressed this concern. Their findings indicated that oxytetracycline leaching varied with water temperature and water pH. In all circumstances, however, leaching did not remove more than 20 percent of the added drug. Perhaps this is only an academic problem as, under practical, well-managed conditions, the fish will rapidly consume the feed given, leaving little time for leaching to occur.

Compatability with feed ingredients is yet another area of concern. Fish feeds, unlike terrestrial animal feeds, contain very high proportions of animal-source ingredient, predominantly fish meal (18). The interaction of antibacterials with different levels of fats and minerals raises questions which may need to be addressed.

Feeding fish in net pens provides the opportunity for excess feed to fall through and build up in the surrounding aquatic or marine environment. The addition of antibiotics to the feed adds another dimension with which to be concerned. Work has been done to examine this possibility (13, 19) and, while residual drugs could be detected and antimicrobial effects could be demonstrated in the sea-farm environment, the changes were not permanent. Nevertheless, management methods will need to be designed with the aim of minimizing pollution of the surrounding environment.

## Tissue Residues

In 1958, the Federal Food, Drug and Cosmetic Act in the United States was amended to require sponsors of new drugs to demonstrate the safety of their products. Safety implied both use-animal and human safety (20). In Canada, similar regulatory procedures were initiated in 1964, with legislation being enacted the following year (21). These regulations, therefore, required an examination of tissues for drug residues and drug depletion times. Thus, from relatively early on, farmers treating their livestock with antibiotics or chemicals have been required to withold those animals from market for specific periods of time.

In Canada, Agriculture Canada is charged with the responsibility of monitoring the food supply for wholesomeness, which includes the absence of drug residues. This program has been highly successful in assuring a safe food supply to the consumer.

The situation in fish culture is somewhat different. In Canada, drug use in fish has been unregulated until recently, but information on drug withdrawal times has been available, at least for some products, since the early sixties. In 1963, investigations were begun by the U.S. Fish and Wildlife Service which resulted in the registration 4 years later of sulfamerazine for use in fish (12). These investigations presumably determined withdrawal times, as required by U.S. law. The earliest reports of depletion times appear to be those of Scandanavian workers, beginning in 1964 with a series of publications showing tissue depletion of sulfamerazine and tetracycline. A 1976 report by McCracken and others (22) gave tissue depletion times for chloramphenicol, framycetin, oxytetracycline, and potentiated sulfadiazine.

Accompanying the boom in salmon culture in Europe there has been a number of reports of residue depletion and analysis methods published. Even today, investigations are continuing on this important area of study in many centers in Europe and North America.

One of the most important concerns to fish producers is the variation in kinetics of drugs in fish with varying water conditions. This makes a precise definition of depletion times extremely difficult. Novel means of defining withdrawal times may be necessary, such as a determination based on degree-days, or times varying with temperature ranges, such as the 40 day/80 day recommendation of WHO. Perhaps the most definitive method lies in the development of rapid residue detection techniques.

The variety of information developed at various centers throughout the world as well as the mounting concerns over residues in farmed fish required some coordination to put everything into perspective and to give direction to the fish farming community and its support groups. This has begun in the form of a working party of the UN Food and Agriculture Organization. This group's meeting resulted in a report by Professor F. Rasmussen (23) which outlined the state of knowledge of therapeutic use in fish production and identified areas of research and regulatory needs. The topic identified as having the most urgent need for research was, not surprisingly, pharmacokinetics of therapeutics in fish. Also identified as urgently required was consistent regulations for drug use, the need for scientifically-based withdrawal periods which are, at the same time, practical, and regulations to protect the environment. The only recommendation proposed by the group was that the general withdrawal period for therapeutics be 40 days after final treatment for fish in water temperatures of 10°C or above and 80 days after final treatment for fish in water temperatures below 10°C.

Clearly, the area of antibiotic use in fish requires, still, a great deal of input to develop logical and useful treatment programs based on ever-greater knowledge of disease processes, feeding programs and other methods to enhance drug delivery, and well-defined withdrawal periods. As time goes on, we will hopefully see new products developed for the aquaculture market which will be totally compatable with fish feeds, and highly efficaceous at low levels to allow rapid tissue clearance.

**TABLE 1 - Feed Additive Antibacterials Used in Agriculture**

| Antibiotic | Synthetic |
|---|---|
| *Bacitracin | *Carbadox |
| *Bambermycins | Furazolidone |
| *Chlortetracycline | Nitrofurazone |
| *Erythromycin | Sulfamethazine |
| Hygromycin B | Sulfathiazole |
| *Lasalocid | |
| *Lincomycin | |
| *Monensin | |
| Neomycin | |
| Novobiocin | |
| Nystatin | |
| *Oxytetracycline | |
| *Penicillin G | |
| *Salinomycin | |
| Streptomycin | |
| *Tylosin | |
| *Virginiamycin | |

* Compounds with growth promoting effect.

Information gathered from references 2, 4, and 14.

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**TABLE 2 - Antibacterials Used as Feed Additives in Aquaculture.**

Chloramphenicol
Erythromycin
Flumequin
Furazolidone
Oxolinic Acid
Oxytetracycline
Piramidic Acid
Potentiated Sulfonamide
Sulfamerazine

Information gathered from references 7, 12, and 21

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# Anti-oxidants in fishmeal and fish feeds - current knowledge and practice

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Anti-oxidants are used in fish feeds to prevent deterioration in the quality of the fish oil and fishmeal caused by oxidation from atmospheric oxygen. The sites of attack are the unsaturated portions of the fatty acid moieties of triglycerides. In general, susceptibility to oxygen increases with increasing unsaturation, the fatty acids mainly involved being those with 3 or more double bonds. Fish oils, and the lipid in fishmeal, are noted for their content of highly unsaturated fatty acids and oxidative deterioration is a greater problem than in most other common oils.

The oxidation of unsaturated fatty acids at ordinary temperatures results initially in the formation of peroxides by an autocatalytic process, that is, the rate of oxidation increases with time. Autoxidation occurs in a 3-stage, free-radical initiated chain reaction viz. (i) initiation (ii) propagation (iii) termination, the initial products are mainly hydroperoxides. These decompose to low molecular weight compounds, and a large number of saturated and unsaturated aldehydes, ketones, acids and other products have been isolated from oxidized oils and shown to be toxic or potentially toxic.

Measurement of oxidation in fishmeal is a problem that has not been satisfactorily resolved. In the early stages of oxidation there is a reasonable correlation between oxidation and hydroperoxide content because in these early stages hydroperoxide formation greatly exceeds its decomposition, later when hydroperoxide decomposition increases, peroxide measurements are

less reliable as quantitative measures of oxidation. Thus measurement of several indicators of peroxidation level such as (i) peroxide value (ii) thiobarbituric acid value (iii) anisidine value and (iv) conjugated double bond systems (spectrophotometrically at 232 nm) is desirable.

For use in fish diets, fish oil should have peroxide and anisidine values of less than 5 mEq/kg and 10 mEq/kg respectively. It should be stabilized by de-aerating and adding synthetic anti-oxidant (ethoxyquin, butylated hydroxy toluene or butylated hydroxy anisole) at the rate of 500 mg/kg and mixing it by bubbling $N_2$ gas. Storage should be in an airtight container. Fishmeal should be protected by addition of ethoxyquin, sprayed on in liquid form during its preparation, at the rate of 200 mg/kg.

Synthetic anti-oxidants are added to dietary components which contain unsaturated oils because the natural, lipid soluble anti-oxidant vitamin E is usually supplemented to diets in ester form (e.g. tocopheryl acetate). This is very stable but has little or no anti-oxidant activity until it is hydrolysed in the gut to tocopherol prior to absorption. Were tocopherol added to the diet, much of it might be oxidized during storage and prior to feeding.

The main pathologies resulting from vitamin E deprivation are muscular dystrophy, edema and haemorrhages arising from increased capillary permeability, exudative diathesis - accumulation of fluid (often greenish in colour from decomposed hemoglobin) under the skin or in the abdominal cavity, ceroid pigment in the liver and an increase in erythrocyte fragility.

Several studies on rainbow trout have shown them comparatively resistant to vitamin E deprivation unless oxidized polyunsaturated fatty acids were included in the diet. At low water temperatures (6°C) vitamin E deficiency

did lead to severe myopathy indicating that maintenance of fluidity in biomembranes is more demanding at low temperatures (Cowey et al. 1984). Conversely nutritional muscular dystrophy and other vitamin E deficiency pathologies have been observed in warmwater fish (channel catfish) given diets containing mainly saturated fat (Lovell et al. 1984).

Protective systems against lipid peroxidation within the fish are (1) enzyme systems that prevent initiation, superoxide dismutase, GSH peroxidase, catalase, caeruloplasmin ($Fe^{2+} \longrightarrow Fe^{3+}$) (2) radical reaction terminators, vitamins E and C, carotenoids, GSH (3) proteins that bind iron, e.g. transferrin. Nutrients, other than tocopherol, with a role in anti-oxidant metabolism, are Se (cofactor for GSH peroxidase), Zn and Cu (cofactors for superoxide dismutase), carotenoids and retinol (radical scavengers in lipid phase), ascorbic acid and riboflavin (coenzymes for GSH reductase).

Aspects of feed formulation that may affect lipid oxidation are addition of iron as $Fe^{3+}$ or organic iron, addition of ascorbic acid as the phosphate (so avoiding reduction of $Fe^{3+}$), use of synthetic antioxidants and of vitamin E in ester form, sufficiency of Se and of sulfur acids for GSH synthesis.

Aspects of husbandry that bear on prevention of lipid peroxidation include (1) conditions of feed storage (2) avoiding effects of strong sunlight (e.g. extruded diets floating on water surface) (3) avoidance of abrasions, surface damage (small molecular weight Fe and other metals are released in dead or damaged tissue, there is a loss of protective mechanisms) (4) any mortalities should be checked for edema and ceroid; obtain erythrocyte fragility test and liver tocopherol concentration.

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# SUMMARY

## Pigmenting Agents in Fish Feed

## Current Knowledge and Practice

Hermann Hencken and

Regula Estermann

The red colour of salmonids is the most decisive quality criterion, differentiating salmonids from a number of other fish species.

The red colour of salmonids has its origin in the red pigmenting carotenoids (astaxanthin, canthaxanthin) contained in the diet.

In the flesh of wild salmon (Figure 1) carotenoids other than astaxanthin are only found in traces. Not until sexual maturity, when astaxanthin is transferred from the flesh to the ovaries and particularly the skin, are other carotenoids formed metabolically from the original astaxanthin.

There are some natural sources of astaxanthin available to the aquaculture industry (Tables 1 and 2).

A real breakthrough with regard to a reliable and standardized source of astaxanthin for the aquaculture industry was the launch of synthesized astaxanthin (Carophyll Pink).

Suggested dietary inclusion levels for various species are given in Table 3. Lower dietary levels with a correspondingly longer feeding period are recommended for a better utilization of the astaxanthin and a more homogeneous pigmentation of the fish.

In general, the degree of pigmentation in salmonids depends largely on the astaxanthin intake with the diet (Figure 2).

However, a number of factors may alter the usual utilization of dietary astaxanthin. These factors are reviewed in Figure 3.

Such dietary factors are the fat content (Figure 4) as well as some quality specifications and the fatty acid composition of the fat.

Increased amounts of astaxanthin in the feed always lead to increased amounts in the flesh, although at very high concentrations there is no longer a corresponding increase in pigmentation (Figure 5 and 6).

Free astaxanthin is better absorbed by salmonids than esterified astaxanthin. This observation refers to both, natural (Figure 7) and synthesized astaxanthin (Figure 8).

Mono-esters appear to be more digestible than diesters. Fatty acids with a longer chain length impair digestibility to a larger extent than those with a shorter chain length.

The same applies to saturated fatty acids, which reduce the absorption of astaxanthin more than unsaturated fatty acids.

Astaxanthin is used in fish feed mainly to pigment the flesh of salmonids, but carotenoids have a number of other biological functions besides pigmentation. A review of these functions is given in Figure 9. Apart from specific functions in the form of carotenoproteins or by its transformation into the vitamin A precursors A1 and A2, astaxanthin acts mainly as an effective biological antioxidant or free radical scavenger in the metabolism of fish.

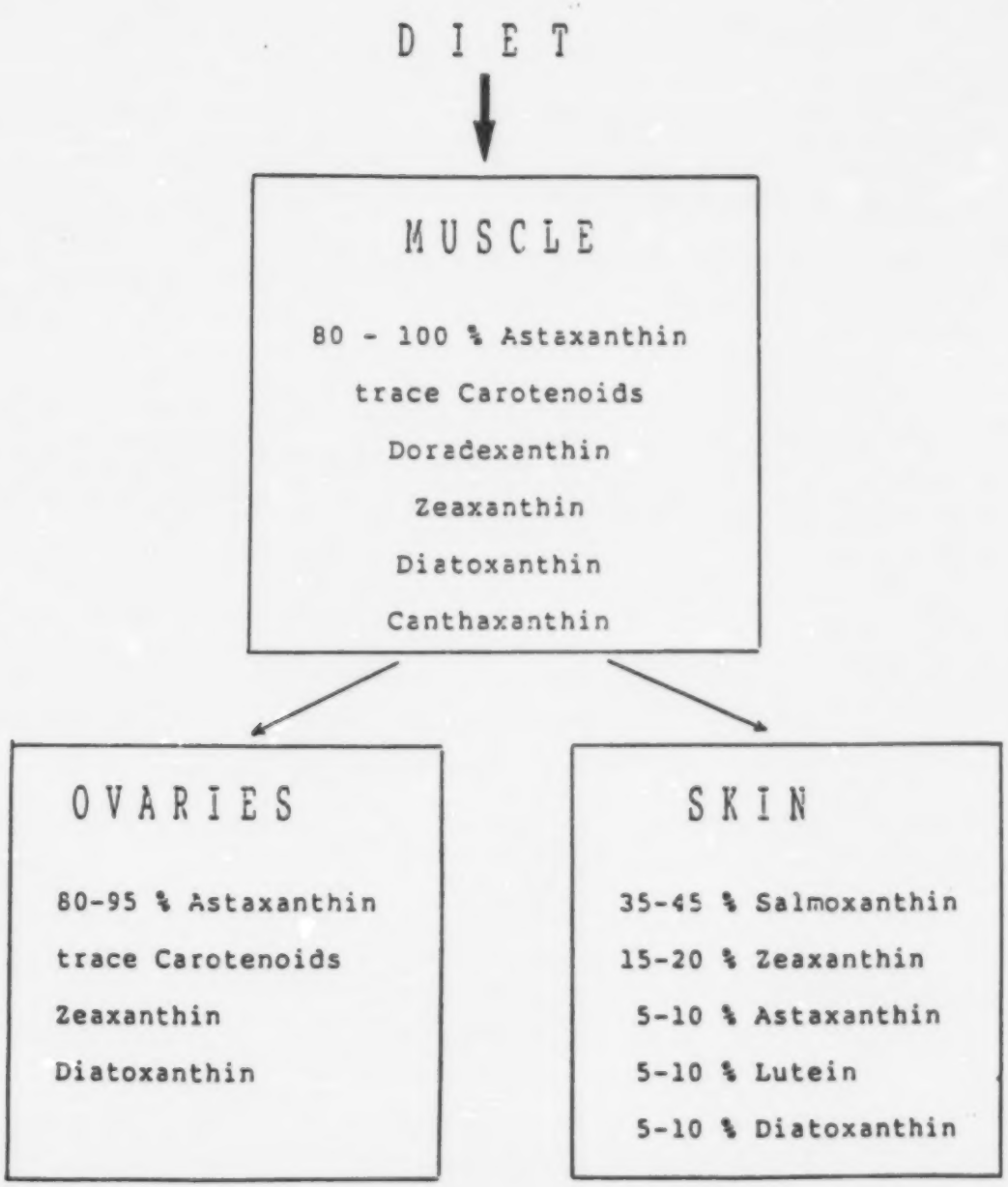
Astaxanthin, like other carotenoids, is sensitive to oxidation, particularly during storage at high temperatures or when exposed to harsh processes in feed manufacturing such as extrusion. Differences between Canada and Europe in extrusion conditions and their influence on the stability of astaxanthin in Carophyll Pink are given in Figures 10 and 11. The higher temperatures and pressure applied during extrusion lead to higher astaxanthin losses in Europe than in Canada.

An important advantage of astaxanthin compared to canthaxanthin is its higher colour stability in the flesh of salmonids during processing (Figure 12). Particularly during smoking, less so during cooking, the trout pigmented with canthaxanthin suffers considerable losses of its original red colour, and changes to a more yellow-brownish hue compared to the fresh trout (Figure 13).

More deeply pigmented salmonids, i.e. those with a higher astaxanthin content in the flesh, loose less "colour" during processing. To deepen pigmentation continuously increasing amounts of astaxanthin in the flesh are required. It is this "built-in reserve" of astaxanthin in the flesh that reduces the extent of colour changes during processing.

Figure 1

# Distribution and Transformations of Carotenoids in Salmon Species






Source: Tadashi Kitahara, 1984

Table 1

# Sources of ASTAXANTHIN

- Yeast: – Phaffia rhodozyma

- Algal Sources: – Haematococcus sp.

- Animal Sources:
  - Shrimp wastes
  - Crayfish wastes
  - Crab wastes
  - Mysis
  - Copepods
  - Krill
  - Rotifers
  - Cuttlefish

Table 2

# ASTAXANTHIN CONTENT OF POTENTIAL CAROTENOID SOURCES

## Astaxanthin Content of Different Crustacean Oil Extracts

| Crustacea | Astaxanthin * (mg/100g Oil) |
|---|---|
| Prawn (Pandalus borealis) | 109,5 |
| Krill (euphasiids) | 72,2 |
| Calanus (C. finmarchicus) | 52,0 |
| Crawfish (Procambarus clarkii) | |
| Soy Oil Extract | 87,4 |
| Herring Oil Extract | 75,2 |
| Menhaden Oil Extract | 72,7 |
| Salmon Oil Extract | 77,8 |

* = Astaxanthin, Astaxanthin-Ester and Astacene

## Astaxanthin Composition of Crawfish Waste and "Pigmented Oil"

| Sample | Astaxanthin % Free | Monoester | Diester |
|---|---|---|---|
| Crawfish Puree | 37,0 | 31,7 | 31,3 |
| Crawfish Press Cake* | 39,4 | 30,6 | 30,0 |
| Pigmented Soy Oil | 12,8 | 45,7 | 41,5 |
| Pigmented Herring Oil | 13,1 | 45,7 | 41,2 |
| Pigmented Menhaden Oil | 13,3 | 44,3 | 42,4 |

"Pigmented Oil": oils used as extraction vehicles for pigment extraction of Crawfish Puree

*Crawfish Press Cake = compressed solid of extraction

Source: T.R. Omara-Alwala et al., 1985

Table 3

# Preferred Dietary Inclusion Rate

**On dry matter basis: 25 - 100 ppm pure astaxanthin**

**depending on target animal and feeding regime**

**Recommended use levels of CAROPHYLL Pink**

| Animal Species | Feeding Period | Astaxanthin pure ppm |
|---|---|---|
| Trout | 3 - 4 weeks | 75 - 100 |
| | 6 - 8 weeks | 50 - 75 |
| | 12 - 14 weeks | 30 - 50 |
| Salmon | 6 - 12 months | 80 - 100 |
| | 12 - 18 months | 40 - 80 |
| Aquarium fish | continuously | 50 - 100 |
| Crustaceans | 8 - 10 weeks prior to harvest | 50 - 100 |
| Reproductive fish | 4 - 8 months prior to spawning | 25 - 75 |

**Please observe any locally authorized inclusion levels !**

Figure 2

# PIGMENTATION OF FISH

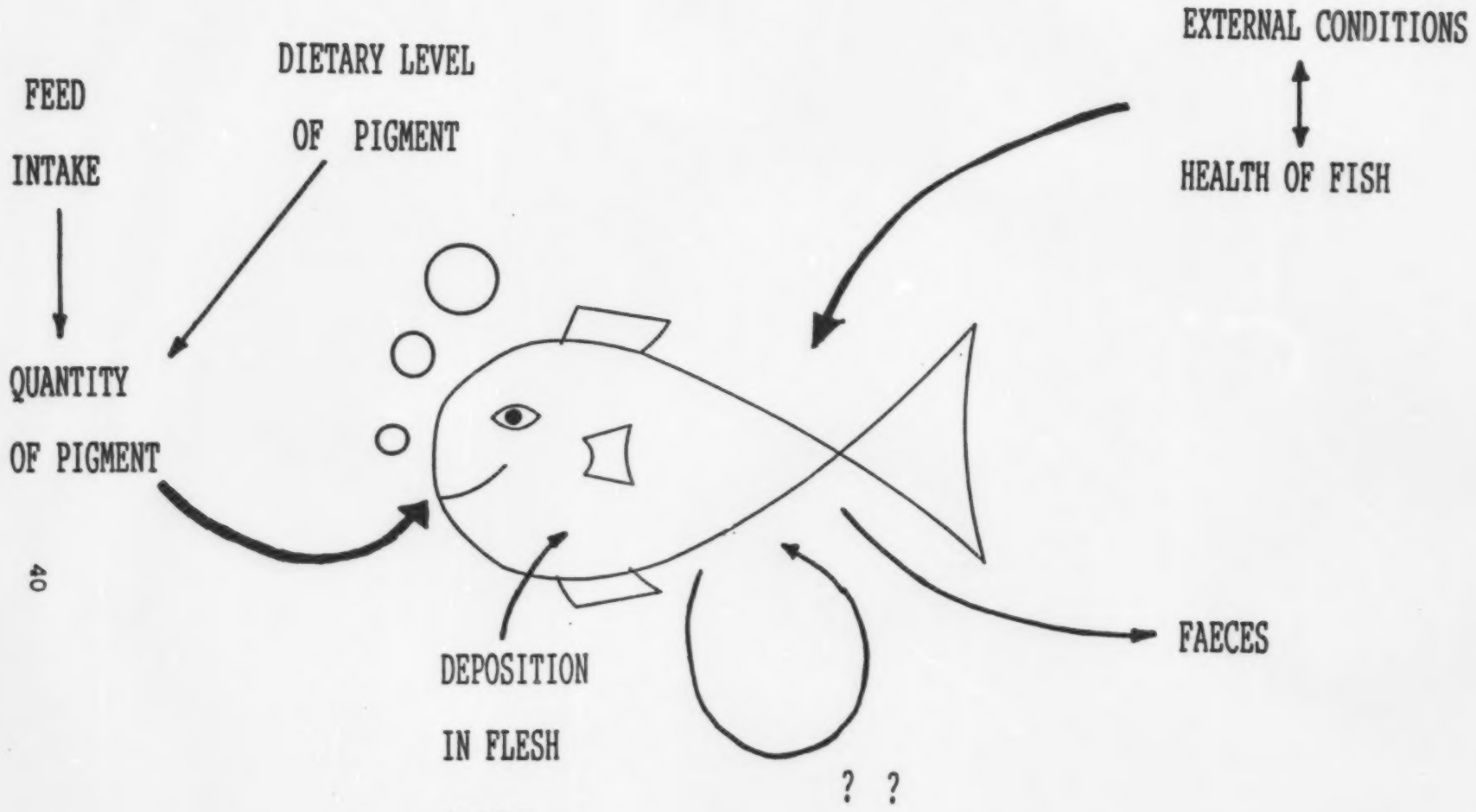


Figure 3

# Factors Influencing Deposition

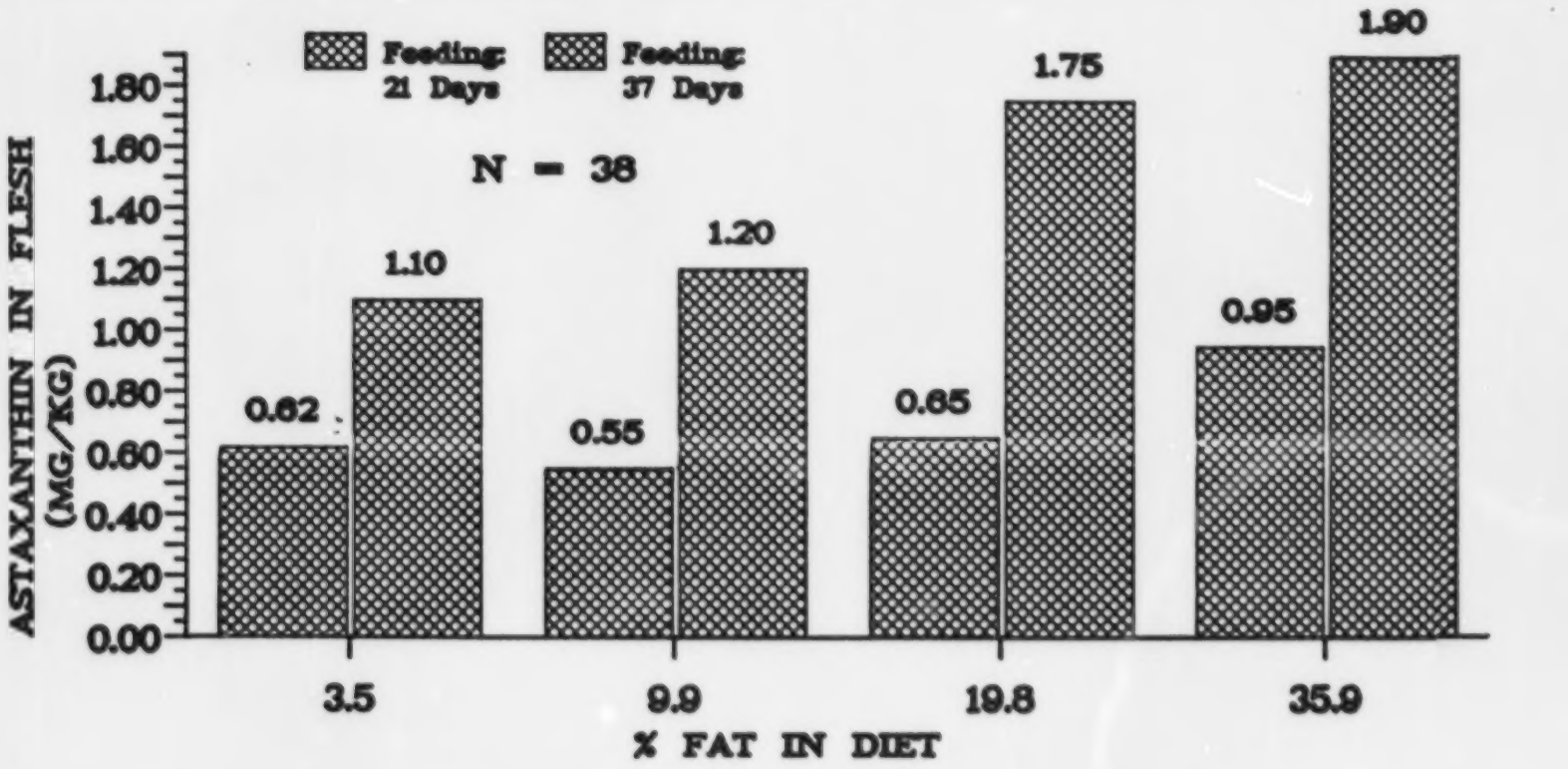
Deposition rate: 5 – 20 %
of ingested ASTAXANTHIN

Deposition rate mainly depends on dietary use level of ASTAXANTHIN and is further influenced by various factors, such as:

- **FEED**
  - Fat Level and Quality
  - Feeding Period
- **FISH**
  - Species
  - Genotyp
  - Sex
  - Maturity
  - Weight
  - Health
- **ENVIRONMENT**
  - Water Quality
  - Temperature
  - Salinity
- **CAROTENOID**
  - Form
  - Digestibility

igure 4

# Effect of Feed Composition (Fat Content) on ASTAXANTHIN Deposition (Rainbow Trout)



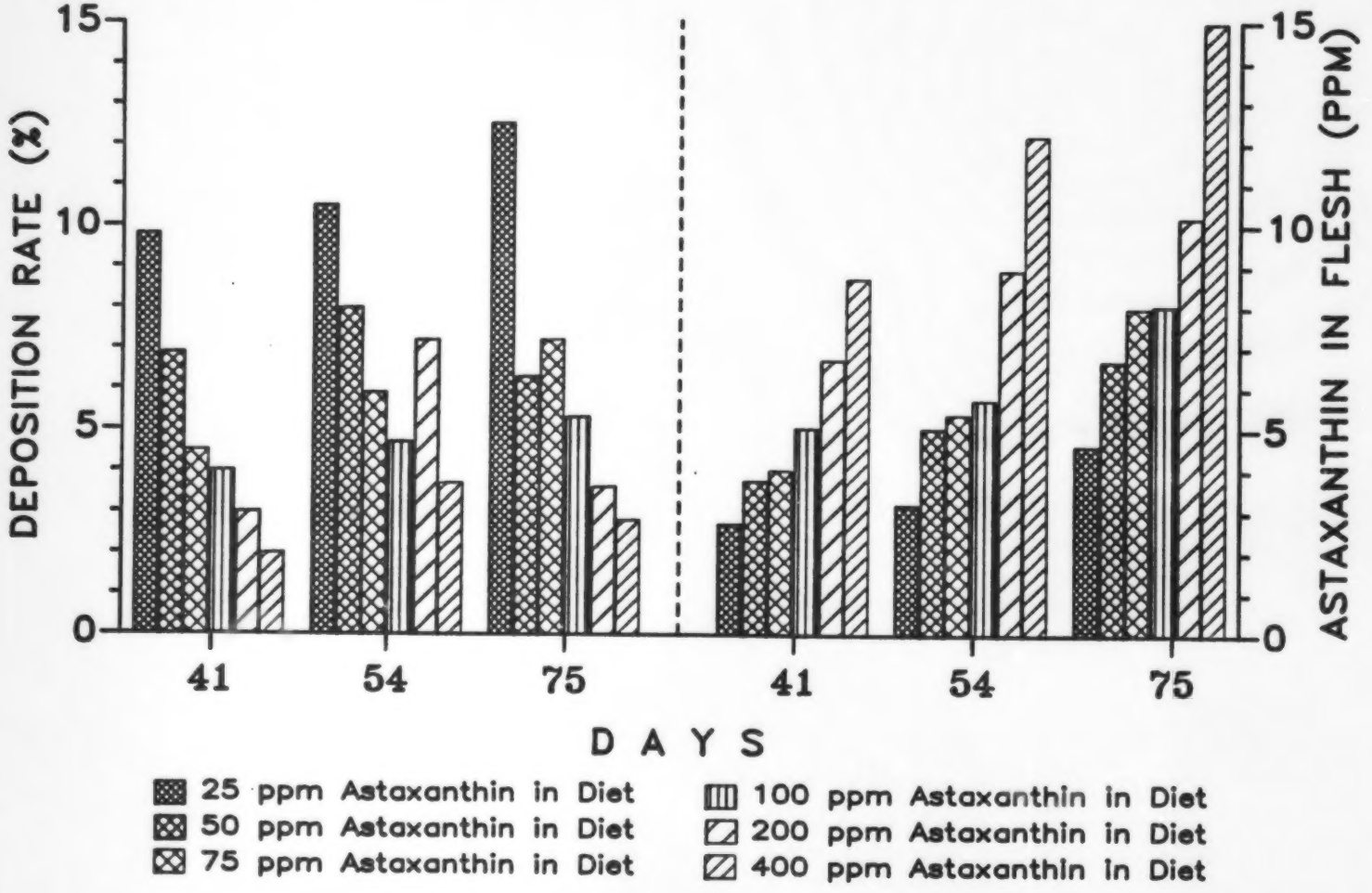

- Fish weight: start trial ⌀ 230g
- Average ASTAXANTHIN content in flesh increases significantly:
  - from start of trial (day 21) to end of trial (day 37)
  - with increasing level of fat in the diet

O.J. Torrissen, 1985

CPD013F/bb

Figure 5

# Effect of Length of Feeding Period on Pigmentation of Rainbow Trout

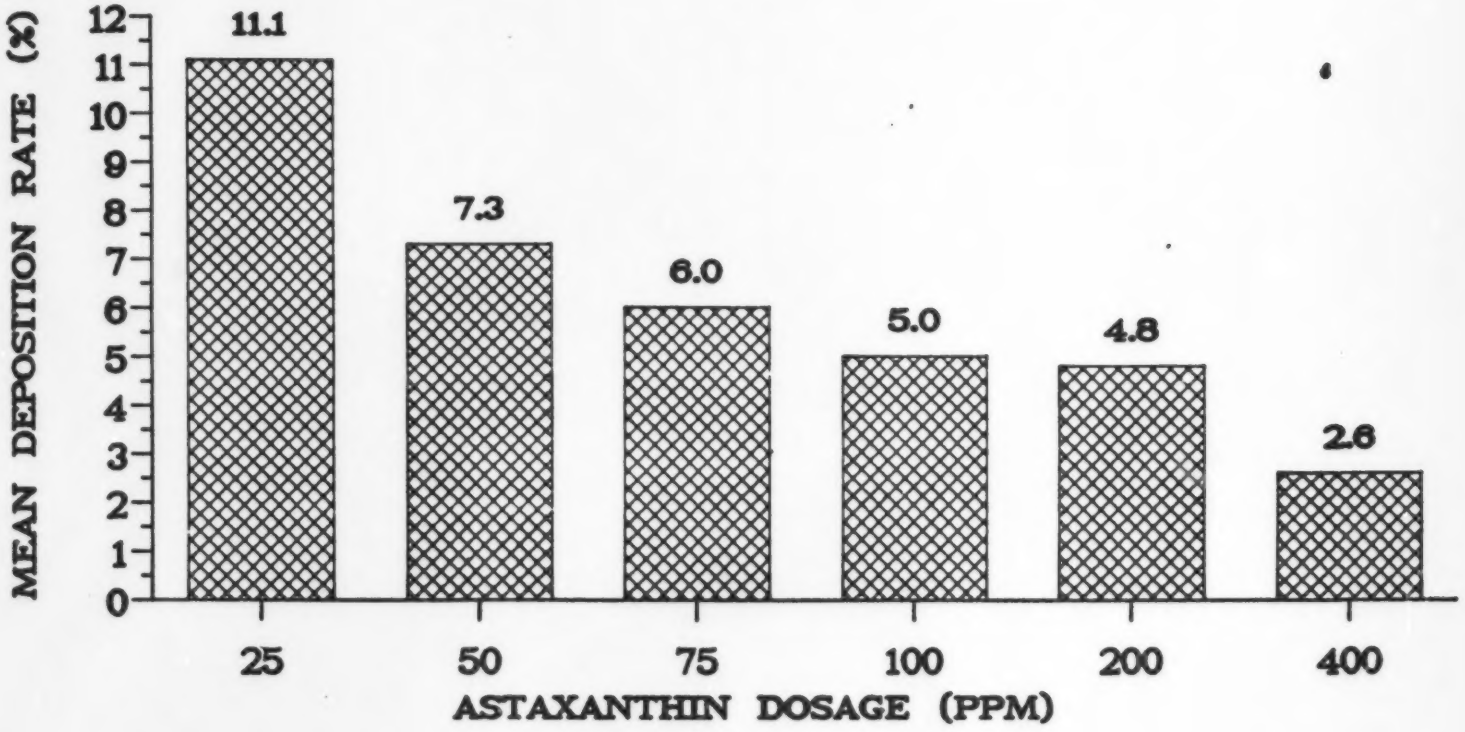



K. Streiff, Basle, unpublished (1984)

REs121/bb

Figure 6

# Mean Dosage Dependent Deposition (%) of ASTAXANTHIN (Rainbow Trout)



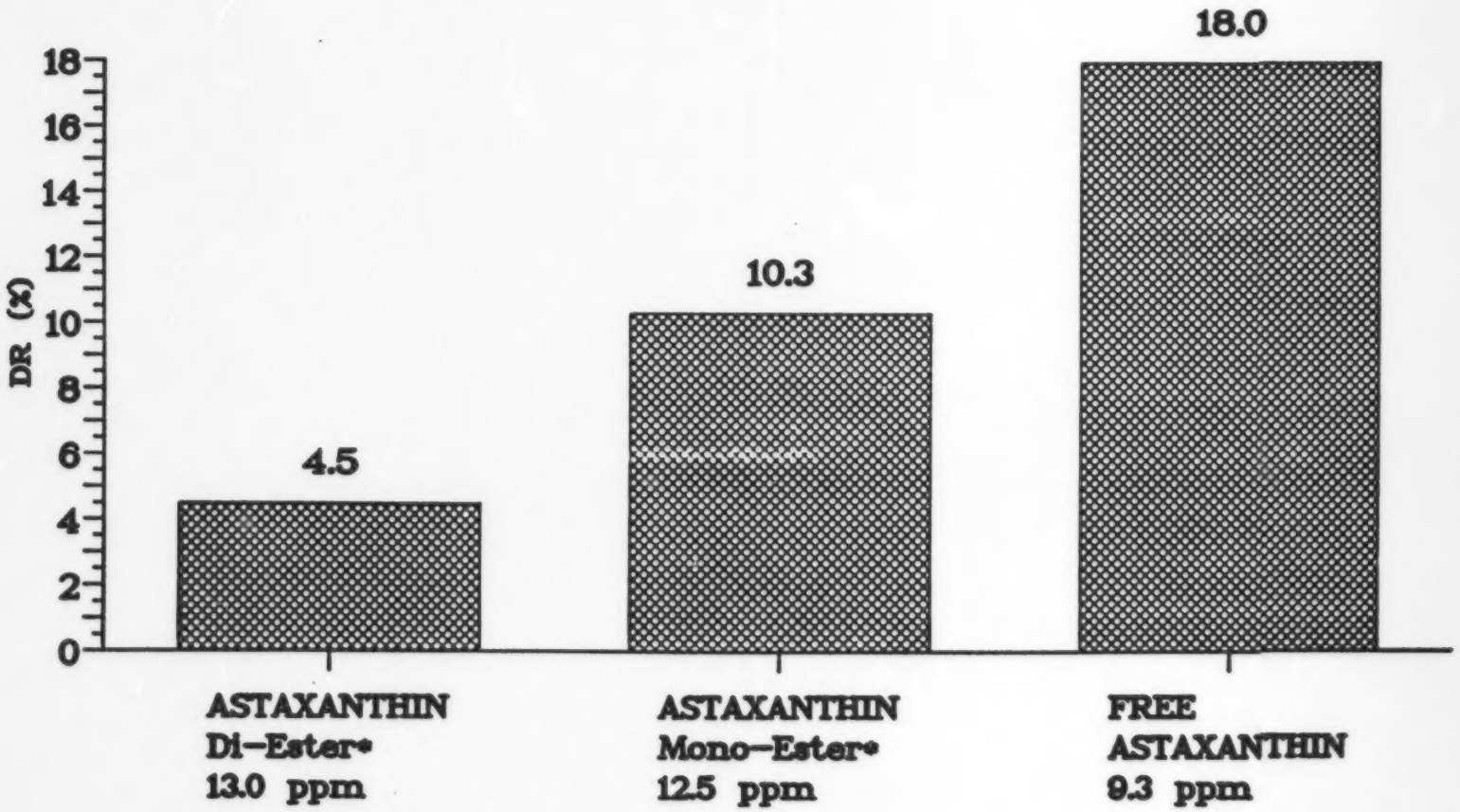

- Diet = Dry Feed Pellets
- Fish weight: start trial ⌀ 150g
  end trial ⌀ 400g

K. Streiff, Unpublished Results Basle, 1987

CPD012F/bb

Figure 7

# Deposition Rate in Rainbow Trout after 33 Days on Different Feeds




* extracted from Calanus, dissolved in oil

– Moist Pellets

O.J. Torrissen, O.R. Braekkan, 1979

CPD034/bb

Figure 8

## Astaxanthin Deposition in Atlantic Salmon (Salmo salar) Fed Different Astaxanthin Forms

Dietary Level of Carotenoids: 50 ppm

Feeding Period: 9 Weeks

| Treatment | Average Level of Carotenoid in Flesh (ppm) |
|---|---|
| Free Astaxanthin | 0.90 |
| Astaxanthin Diacetate | 0.72 |
| Astaxanthin Dipalmitate | 0.28 |



Hoffmann-La Roche, Basle, 1984

Figure 9

# Biological Functions of Pigmenting Carotenoids in Fish / Salmonids

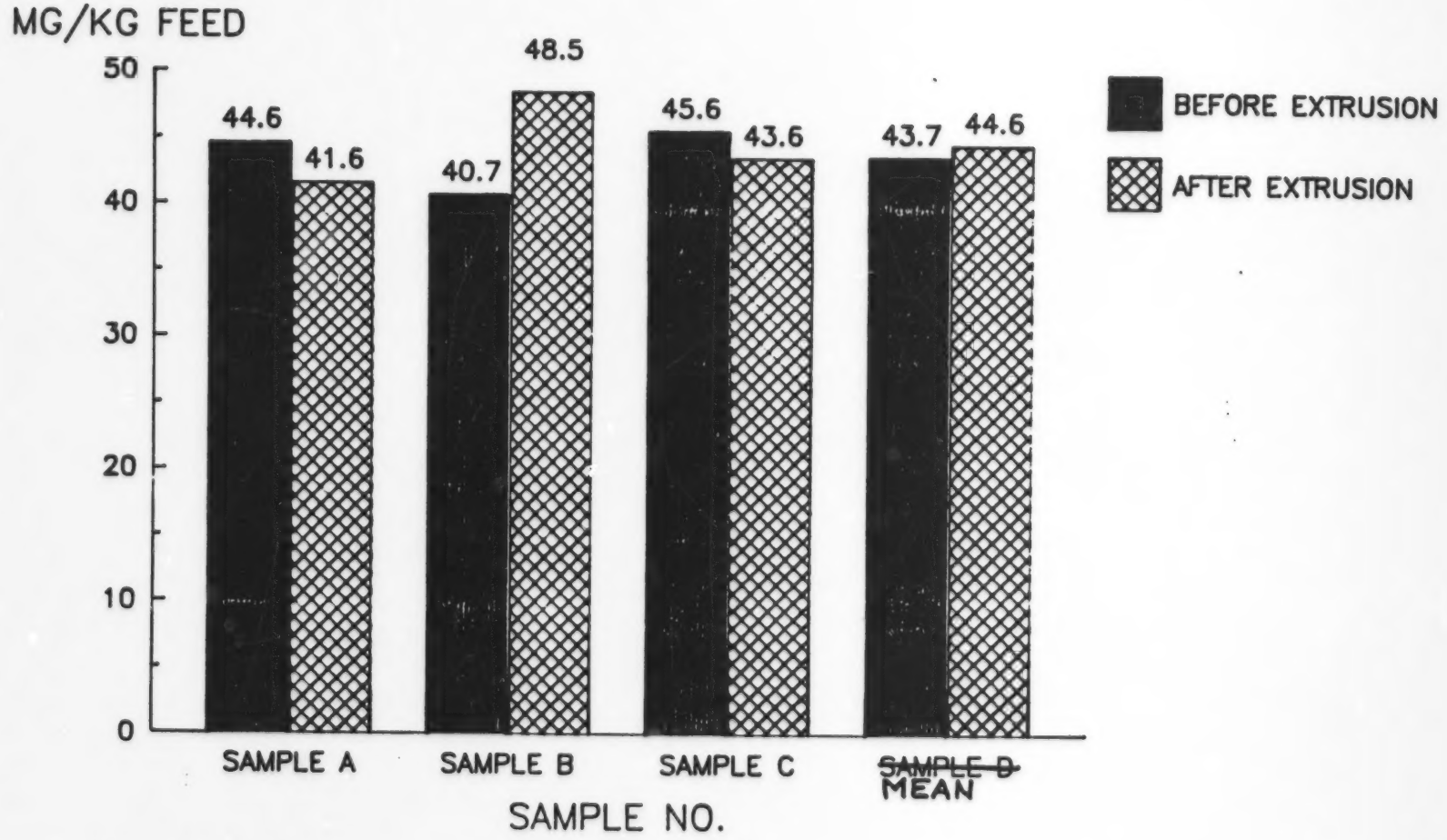
- As colour attractant in the skin at breeding
- As a protective agent in eggs against harmful light, elevated temperature, metabolic products of adult fish and low oxygen tension, thus reducing mortality during embryonic development
- As agent improving the number of normal hatchings
- In protective skin pigmentation (camouflage) of first feeding fish, resulting in higher survival rates
- In improving the growth of fish
- As agent improving the liver performance
- As vitamin A1 and A2 precursors in vitamin A deficient diets
- In the maintenance of an accessory endogenous respiratory system

## Under evaluation:

- As agent improving the immunological system, resulting in an enhanced resistance against diseases
- As agent improving the maturation of oocytes and sexual maturation
- As fertilization improving agent, resulting in a higher proportion of fertilisized eggs

Figure 10

# ASTAXANTHIN* CONTENT OF FISH FEED BEFORE AND AFTER EXTRUSION**

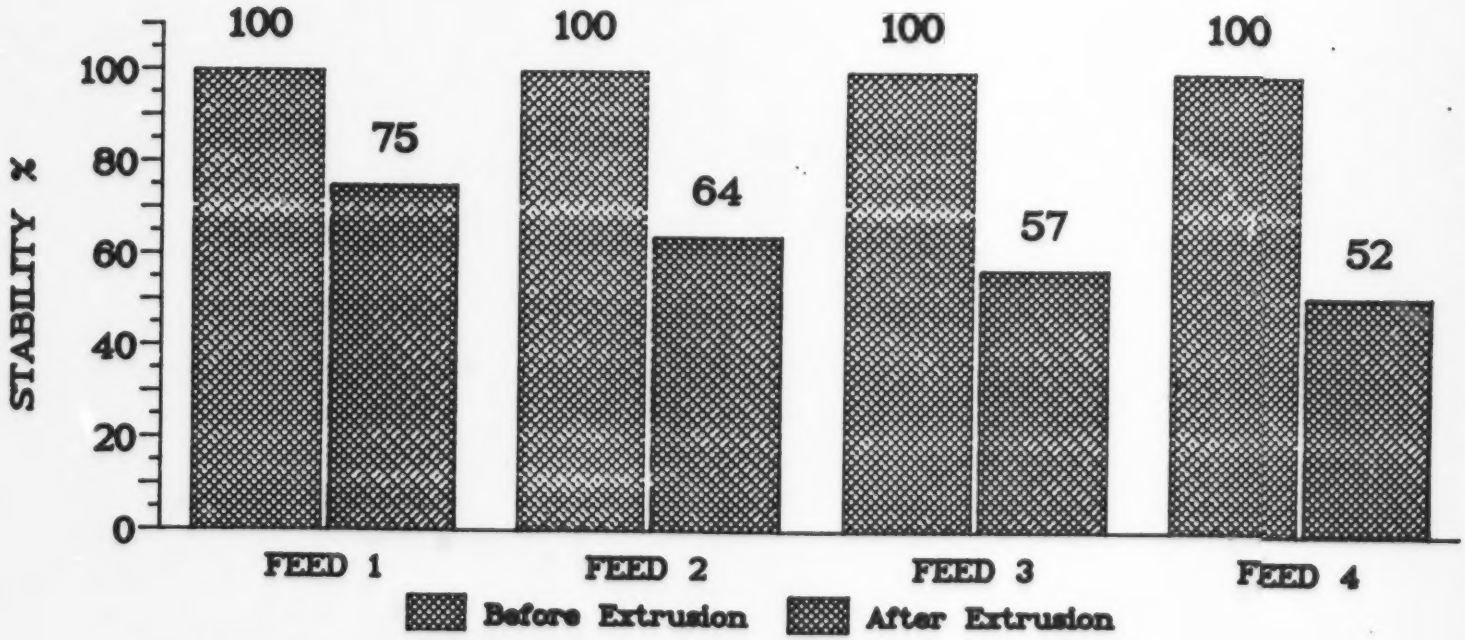



* FROM 'CAROPHYLL' PINK
** CANADIAN DATA

ASTAX.DJB

igure 11

# Stability of ASTAXANTHIN in Fish Feed after Extrusion




- stability (%) varies largely within samples of different origin
- thus extrusion conditions (temperature, pressure) seem not be identical

==> this shows clearly what may happen to a product if it is treated in a different way

Scandinavia, Unpublished Results, 1987

CPD004/bb

Figure 12

# ASTAXANTHIN :

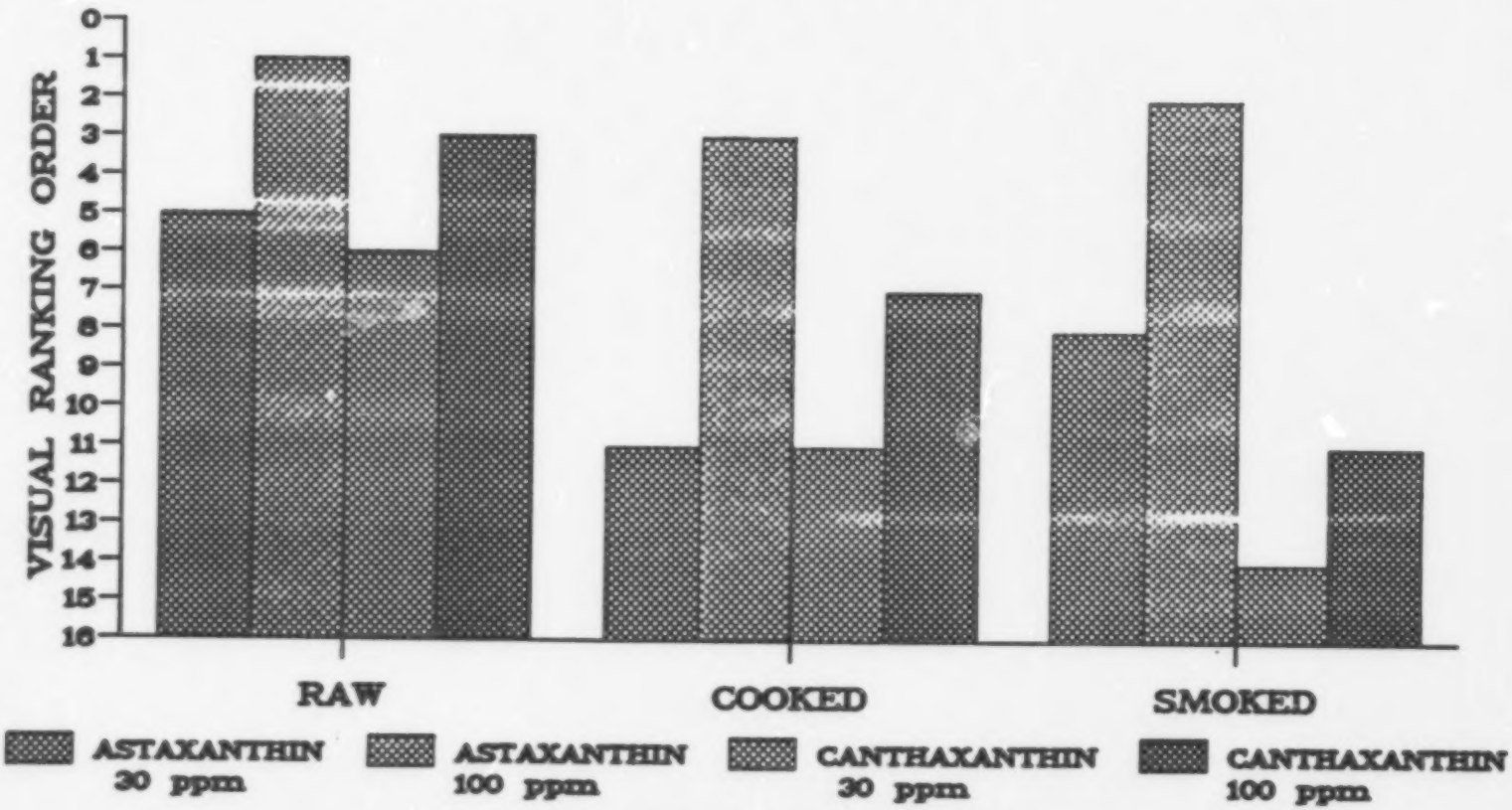
## Its Colour Stability during Processing of Fish

- ASTAXANTHIN pigmented fish shows a better colour stability during processing than CANTHAXANTHIN pigmented fish

- CANTHAXANTHIN pigmented fish has a yellowish touch already at a raw state which becomes even stronger during processing whilst ASTAXANTHIN pigmented fish remains pink

- The stability of ASTAXANTHIN during storage of packed fish depends largely on the chosen packaging and storage conditions

# Colour Stability of ASTAXANTHIN in Fish (Rainbow Trout) during Processing (Raw–Cooked–Smoked)




ppm in Feed (Dry Pellets)

Visual Ranking Order: 1 = Maximum Red
16 = White

K. Streiff, Unpublished Results, Basle, 1979

CPD007a/bb

Summary Discussions

At the end of the meeting, small groups were set up to discuss the six subject areas of the meeting. General conclusions were drawn and topics for further research or definition were suggested.

**Lipid Nutrition Quantity and Quality**
**Summary Presented by Dave Higgs**

Protein:Lipid Ratios

Optimum protein:lipid ratio in different diets eg grower vs broodstock diets should be investigated. There is also a need to determine these ratios for the different pacific salmon species under culture - coho and chinook (and more recently sockeye).

To date we have extrapolated or used information for small salmonids reared in freshwater at more or less constant temperature to derive formulations for large salmon grown out in sea water. It is essential that further work work be undertaken in different sizes of fish in seawater in temperatures ranging from 5-21 degrees C.

Alternative Dietary Lipid Sources

There is a need to find lipid sources alternative to those of marine derivation. In addition to considerations of cost saving, such lipids must be evaluated in terms of: feed efficiency, growth rate, quality of final product - for the fresh market, frozen market and smoked product. Sensory evaluation of the product is an essential component of any lipid evaluation study. It is also critical that the omega-3 fatty acid content of farmed fish be maintained at levels equivalent to those of wild salmon.

Manipulation of Tissue Lipids

Manipulation of tissue lipid composition in market fish should be investigated. Considerations would be total lipid levels as well as total amounts of n-3 and n-6 and their relative proportions. Lipid quality and quality should also be considered when determining the use of the product eg fresh vs frozen and keeping quality or whether the product is to be smoked.

Influence of Diet on Health

There is a great deal of work to be done in the area of nutritional status and dietary influence on fish health.

**Ascorbic Acid - Known and Hypothesized Functions, Effective Dietary Supplementation**
**Summary Presented by Jim Brackett**

Ascorbic Acid Requirements

Two requirement levels appear to exist and need further investigation. One is for prevention of deficiency signs - scoliosis, lordosis etc. The other is for optimal production and disease resistance. There is a strong feeling among some farmers that ascorbic acid is required well in addition to absolute requirements to increase productivity and disease resistance. There is a great deal of anecdotal information to this effect.

Ascorbic Acid Forms

Suppliers must objectively demonstrate the comparative efficiency and cost-effectiveness of the various forms of ascorbic acid available.

Field Trials

Work should be undertaken to demonstrate under controlled conditions the benefits (or lack of) higher ascorbic acid supplementation than that now generally accepted. Such an investigation should include the rearing stage of the animal including pre-smolt and consider "therapeutic" applications such as during times of vaccination, during times of expected disease and other times of additional stress.

Lab and In Vitro Studies

There are problems with extrapolation of lab work to the field however conditions under which an increased ascorbic acid requirement may be expected could be investigated. Certainly effects of supplementation on growth, disease resistance and immune response could be investigated in the laboratory and in vitro.

Assay Techniques

There is a need to standardize techniques for feed and tissue analysis. In addition there should be discussion and determination of what exactly tissue levels mean.

**Antibiotics - Fish Nutrition and Feeding Issues**
**Summary Presented by Don French**

A great amount of work is required in this area. Including the following.

"Approved" inexpensive rapid residue detection techniques. Approved refers to acceptance by the relevant government authorities so that the test can be used to declare that a lot or shipment complies with standards (see below).

Tolerance levels for specified antibiotics are essential to provide industry with guidance and consumers with confidence in the product. Tolerance levels must conform to or exceed acceptable international levels.

Research is needed on optimization of antibiotic delivery techniques. Considerations are feed formulations - can antibiotic uptake be optimized with special feeds? Should feeding regimes - frequency, meal size etc. be modified to optimize uptake? Can the antibiotic formulation itself be modified to enhance its uptake in the salmon feed or in the salmon gut?

Pharmacokinetics must be studied in cold-blooded fish such as salmonids to gain an appreciation of how they handle and "compartmentalize" antibiotics and their derivatives.

Greater understanding of the pathogenesis of the major diseases is required to develop suitable antibiotic therapies.

Withdrawal times must be determined which are useful to the farmer and reliable in terms of consumer confidence and safety. These must be drug specific and consider water temperature.

Alternative therapeutants for treatment of individual diseases must be identified or developed. Greater specificity should be achieved rather than treating a range of diseases with one or two major antibiotics.

The relationship between nutrition and fish health should be investigated.

**Antioxidants in Fish Meal and Fish Feeds - Current Knowledge and Practice**
**Summary Presented by Fred Ming**

Oils

Methods for stabilization in processing and storage should be further investigated and refined so that best use can be made of a finite resource of marine oils. Measurements of oxidation should be refined and standardized.

Meal

Stabilization methods should focus on protection of the epsilon group of lysine and the sulfur group of methionine and cystine.

Finished Feed

The storage implications concerning stability of lysine, methionine and cystine and vitamins should be investigated.

**Carbohydrate in Fish Feeds**
**Summary Presented by Dick Beames**

The main problem with carbohydrate in fish feeds is gut enzyme inadequacies followed by a limited ability to utilize the digested sugars. Hilton and Mazur reported that there is none or little increase in enzyme activity with length of feeding in rainbow trout and chinook salmon respectively. There is a possibility for selection of increased tolerance or utilization of carbohydrate based on differences between strains in glucose tolerance and carbohydrate utilization.

Several experiments to study rate of absorption were suggested.

1. Testing different complexities of carbohydrate.

2. Frequency of feeding.

3. Gelatinization.

4. Effect of hindgut residues.

**Pigmenting Agents in Fish Feeds - Current Knowledge and Practice**
**Summary Presented by Ian McCallum**

Despite recent progress in knowledge and practice of pigmentation and the availability of synthetic astaxanthin to replace or supplement canthaxanthin, more experiments and feeding protocols should be undertaken.

1. Efficiency of Deposition
   -losses during metabolism
   -elucidation of metabolic pathways

2. Stability of Astaxanthin in Fish During Storage
   -determination of optimal storage conditions
   -effect of lipid level and type
   -tissue fat effect on visual perception of colour

3. Alternatives to Synthetics
   -yeasts, crustaceans etc.

4. Feeding Strategies
   -optimal dietary level
   -time fed etc.

5. Species and Strain Differences
   -Chinook strains
   -sockeye, coho etc.

# PROGRAMME

## WEDNESDAY, NOVEMBER 15

19:30 - 22:00 — Reception in Hospitality Suite
Room #551, Laurel Point Inn

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## THURSDAY, NOVEMBER 16

08:45 - 9:00 — Welcome and Introductory Comments

### Session I

09:00 - 10:30 — ASCORBIC ACID - KNOWN AND HYPOTHESIZED FUNCTIONS, EFFECTIVE DIETARY SUPPLEMENTATION

**Review Speaker:** Tom Lovell, Department of Fisheries and Allied Aquacultures, Auburn University

**Vitamin C Polyphosphate: Stability and Bioavailability in Aquaculture Feeds**
Blake Grant, Rangen Aquaculture Resource Centre

**Recent Information on the Use of Ascorbate-2-Sulphate in Fish Feeds**
Hakan Svensson, Ewos, Sweden

10:30 - 11:00 — Coffee Break

### Session II

11:00 - 12:30 — LIPID NUTRITION - QUANTITY AND QUALITY

**Review speaker:** Daniel P. Selivonchick, Oregon State University

**Proximate and Lipid Composition of Wild and Farmed Pacific Salmon**
Bakhshish Dosanjh and Dave Higgs, DFO, West Vancouver

**Soft Flesh Syndrome - Apparent Lack of Correlation with Lipid Levels in Farmed Chinook Salmon**
Derek Monteith, B.C. Research

**Observations of Dietary Influence on Lipid Characteristics of Chinook Salmon Eggs**
Dave Groves and Heather Ashton, Seaspring Salmon Farms

12:30 - 13:30 Hosted Lunch

## Session III

13:30 - 15:30 ANTIBIOTICS - FISH NUTRITION AND FEEDING ISSUES

**Review Speaker:** Bill Cox, Pfizer Canada

**Antibiotics and Feeding During Disease**
Jim Brackett,Syndel Laboratories

**Stability of Oxytetracycline Through the Feed Milling Process**
Jack Markert, Moore-Clark

**Development of Assay Methods for the Determination of Romet 30 and Oxytetracycline in Pacific Salmon**
Keith McErlane, U.B.C.

**Development of Rapid Techniques for the Detection of Antibiotic Residues in Salmon Flesh**
Steve Newman, Microtek, Micrologix

**Use of Charm Methodology for Rapid Detection of Antibiotic Residues**
Bruce Burton, B.C. Research

15:30 - 16:00 Coffee Break

## Session IV

16:00 - 17:00 ANTIOXIDANTS IN FISH MEAL AND FISH FEEDS - CURRENT KNOWLEDGE AND PRACTICE

**Review Speaker:** Colin Cowey, University of Guelph

17:00 Close of day's sessions

19:30 Chinese Banquet - Hunan Village, Victoria

FRIDAY, NOVEMBER 17

## Session V

9:00 - 10:00 — CARBOHYDRATE IN FISH FEEDS

**Review Speaker:** John Hilton,
Hoffmann-La Roche Limited,
Mississauga, Ontario

**Glucose Tolerance in Selected Strains of Chinook Salmon**
Mazur et al, Department of Animal Science, U.B.C.

10:00 - 10:30 — Coffee Break

## Session VI

10:30 - 11:30 — PIGMENTING AGENTS IN FISH FEEDS - CURRENT KNOWLEDGE AND PRACTICE

**Speaker:** Dr. Herman Hencken,
Hoffmann-La Roche Limited,
Basle, Switzerland

**Use of Krill as a Natural Pigment and Omega - 3 Fatty Acid Source for Farmed Salmon**
Michael Roch, Retech Ltd.

## Session VII

11:30 - 12:30 — OPEN SESSION

This session is open to papers of general interest, for discussion on research directions, etc.

12:30 — Close of Meeting

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